(2)

OFFICE OF NAVAL RESEARCH

GRANT N00014-90-J-1943

R&T Code 4134050

Technical Report No. 4

Surface-Induced Dissociation of Multiply-Protonated Peptides

by

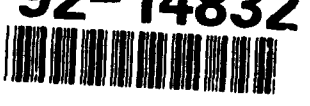
Ashley L. McCormack, Jennifer L. Jones, Vicki H. Wysocki

Prepared for Publication

in the

Journal of the American Society for Mass Spectrometry

Virginia Commonwealth University
Department of Chemistry
Richmond, VA



REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED

4. TITLE AND SUBTITLE
Surface-Induced Dissociation of Multiply-Protonated Peptides

5. FUNDING NUMBERS
G N00014-90-J-1943

6. AUTHOR(S)
Ashley L. McCormack, Jennifer L. Jones, Vicki H. Wysocki

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Department of Chemistry
Virginia Commonwealth University
Richmond, VA 23284-2006

8. PERFORMING ORGANIZATION REPORT NUMBER
4

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
Chemistry Program
800 N. Quincy ST
Arlington, VA 22217

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release
distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

We report here surface-induced dissociation spectra of three multiply-charged peptides: doubly-protonated angiotensin I, doubly-protonated renin substrate, and triply-protonated mellitin. For comparison, the collision-activated dissociation spectra of renin substrate and mellitin are also presented. The spectra show that surface-induced dissociation provides structural information on multiply-charged peptides at sample concentrations compatible with electrospray ionization. For angiotensin I, renin substrate, and mellitin, surface collisions (100-165 eV) favor a limited number of fragmentation pathways, which are the same as those favored in collision-activated dissociation experiments.

14. SUBJECT TERMS			15. NUMBER OF PAGES
			16. PRICE CODE
17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
unclassified	unclassified	unclassified	

GENERAL INSTRUCTIONS FOR COMPLETING SF 298

The Report Documentation Page (RDP) is used in announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to ***stay within the lines*** to meet ***optical scanning requirements***.

Block 1. Agency Use Only *(Leave blank)*.

Block 2. Report Date. Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year.

Block 3. Type of Report and Dates Covered. State whether report is interim, final, etc. If applicable, enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

Block 4. Title and Subtitle. A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, add volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

Block 5. Funding Numbers. To include contract and grant numbers; may include program element number(s), project number(s), task number(s), and work unit number(s). Use the following labels:

C	- Contract	PR	- Project
G	- Grant	TA	- Task
PE	- Program Element	WU	- Work Unit Accession No.

Block 6. Author(s). Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

Block 7. Performing Organization Name(s) and Address(es). Self-explanatory.

Block 8. Performing Organization Report Number. Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es). Self-explanatory.

Block 10. Sponsoring/Monitoring Agency Report Number. *(If known)*

Block 11. Supplementary Notes. Enter information not included elsewhere such as: Prepared in cooperation with..., Trans. of...; To be published in.... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

Block 12a. Distribution/Availability Statement. Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NOFORN, REL, ITAR).

- **DOD** - See DoDD 5230.24, "Distribution Statements on Technical Documents."
- **DOE** - See authorities.
- **NASA** - See Handbook NHB 2200.2.
- **NTIS** - Leave blank.

Block 12b. Distribution Code.

- **DOD** - Leave blank.
- **DOE** - Enter DOE distribution categories from the Standard Distribution for Unclassified Scientific and Technical Reports.
- **NASA** - Leave blank.
- **NTIS** - Leave blank.

Block 13. Abstract. Include a brief *(Maximum 200 words)* factual summary of the most significant information contained in the report.

Block 14. Subject Terms. Keywords or phrases identifying major subjects in the report.

Block 15. Number of Pages. Enter the total number of pages.

Block 16. Price Code. Enter appropriate price code *(NTIS only)*.

Blocks 17. - 19. Security Classifications. Self-explanatory. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

Block 20. Limitation of Abstract. This block must be completed to assign a limitation to the abstract. Enter either UL (unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

Communication Submitted to *J. Am. Soc. Mass Spectrom.* 2/92

Surface-Induced Dissociation of Multiply-Protonated Peptides

Ashley L. McCormack[§], Jennifer L. Jones, Vicki H. Wysocki*
Department of Chemistry
Virginia Commonwealth University
Richmond, Va 23284-2006

* Author to whom correspondence should be addressed
§ Present address: Division of Biology, 139-74, California Institute of Technology Pasadena, CA 91125

Abstract

We report here surface-induced dissociation spectra of three multiply-charged peptides: doubly-protonated angiotensin I, doubly-protonated renin substrate, and triply-protonated mellitin. For comparison, the collision-activated dissociation spectra of renin substrate and melittin are also presented. The spectra show that surface-induced dissociation provides structural information on multiply-charged peptides at sample concentrations compatible with electrospray ionization. For angiotensin I, renin substrate, and mellitin, surface collisions (100-165 eV) favor a limited number of fragmentation pathways, which are the same as those favored in collision-activated dissociation experiments.

In the case of singly protonated peptides produced by liquid secondary-ion mass spectrometry, gas-phase collisional excitation of large peptides (<3000 Da) produces sufficient fragmentation for structural analysis [1]. Extension to larger peptides is limited by losses in desorption and ionization efficiency and by the partitioning of a limited amount of internal energy to a large number of vibrational modes. Electrospray ionization has gained considerable attention because it is an efficient means of generating multiply-charged ions from large biomolecules, including proteins [2-6]. Although the fragmentation mechanisms for formation of multiply-charged peptides have not been fully elucidated, low-energy gas-phase collisional activation of mass-selected multiply-protonated peptides has been shown to provide structural information [7]. Collision-activated dissociation of peptides between the ESI skimmer cone and capillary can provide an additional method for obtaining sequence information [8,9]. Surface-induced dissociation (SID) is an alternative means of dissociating ions; several investigators have reported SID spectra of singly-charged peptides produced by liquid-secondary ion mass spectrometry [10-13]. We report here surface-induced dissociation spectra of multiply-charged peptides and compare the spectra with those obtained by collision-activated dissociation.

Experimental

The instruments used in this investigation are a simple, inexpensive dual quadrupole mass spectrometer specifically designed for ion/surface studies [14] and a triple quadrupole mass spectrometer (Finnigan TSQ70). Experimental details for the triple quadrupole mass spectrometer have been reported previously [15]. The SID instrument consists of two Extrel quadrupoles (m/z range 0-4000 Da) arranged at 90°, with a surface

positioned to intersect the ion optical path of each quadrupole. The angle of the incident beam is 50° with respect to the surface normal. The surface used in this investigation was stainless steel although alternative surfaces are being investigated [16]. Data were acquired and processed with a Teknivent/Vector Two data system.

Electrospray ionization on the SID instrument was accomplished by using a modified version of the recently published electrospray designs of Chowdhury, Katta, and Chait [17] and Papac, Schey, and Knapp [18]. The samples were dissolved in a 45:45:5 (v/v/v) water/methanol/acetic acid solution at final concentrations of 10-50 pmol/μL. Samples were sprayed, with a syringe pump, through a syringe needle (4-5 kV) toward a metal capillary (170-200 V) at a rate of 2 μl/min. A heater wire in fiberglass sleeving is wrapped around the metal capillary to thermally desolvate the ions. The multiply-protonated peptides were mass-selected by Q1 and allowed to collide with the surface at a selected laboratory collision energy. The product ions were analyzed by Q2. The laboratory collision energy is determined by (i) the potential difference between the skimmer cone and the surface and (ii) the charge state of the ion. For simplicity, the potential difference between the skimmer cone and surface will be listed as ΔV; the kinetic energy of the collision is determined by multiplying ΔV by the charge state. Good quality SID spectra can be obtained by averaging data for a total sample spray time corresponding to < 500 picomoles; this is higher than the low picomole levels (10-50 picomoles) required for FAB/SID [19] and thus may reflect sample loss prior to Q1, rather than losses in the activation step.

Results

Surface-induced dissociation spectra are shown below for three peptides: doubly-protonated angiotensin I, doubly-protonated renin substrate, and triply-protonated mellitin.

The peptides vary in average molecular mass from 1298 to 1760 to 2848, respectively. For comparison, the collision-activated dissociation spectra of renin substrate and melittin are also presented.

The surface-induced dissociation spectrum obtained for doubly-protonated angiotensin I is shown in Figure 1 (m/z 649=$(M+2H)^{++}$; ΔV=50). Extensive fragmentation occurs upon SID and results in mainly singly-charged product ions. The series of b-type ions detected allows the assignment of residues 3 to 6. Dominant immonium ions from the tyrosine, histidine, and proline residues are also detected and are indicative of the high energy deposition associated with SID. A systematic investigation of the influence of molecular size and collision energy on the SID fragmentation of singly-charged peptides has shown that, at a given collision energy, the ratio of abundances of high-mass ions to low-mass ions increases with an increase in the size of the peptide [19].

The surface-induced dissociation spectrum obtained for a larger doubly-protonated ion, renin substrate, is shown in Figure 2a (m/z 881=$(M+2H)^{++}$; ΔV=50). An increase in the abundance of high mass ions with an increase in molecular weight is noted (c.f., Figure 1 and Figure 2a). The series of b-type ions allow the assignment of residues 3-6 and 9 and the series of doubly-charged b-type ions allow the assignment of residues 7-12. Several immonium ions are also detected. No y-type ions are present in the spectrum, which is reasonable because the most basic amino acid is located near the N-terminus of the peptide. For comparison, the CAD spectrum obtained at ΔV=30 for the $(M+2H)^{++}$ ion, m/z 881, generated from renin substrate is shown in Figure 2b. The CAD spectrum is remarkably similar to the SID spectrum. It exhibits a series of b-type ions, which allows the assignment of residues 3-6 and 9-10, and a series of doubly-charged

b-type ions, which allows the assignment of residues 7-13.

The surface-induced dissociation spectrum for a triply-protonated, 27-residue peptide, melittin, is shown in Figure 3a (m/z 950=$(M+3H)^{+++}$; ΔV=55). A series of b-type ions allows the assignment of residues 3-5 and a series of doubly-charged y-type ions allows the assignment of residues 5-9, 12 and 13. Again, low mass ions are of greater abundance than high mass ions. For comparison, the CAD spectrum obtained at ΔV=30 for the $(M+3H)^{+++}$ ion, m/z 950, generated from melittin, is shown in Figure 3b. Essentially the same ions are present as those detected in the SID spectrum. Interestingly, the two spectra of Figure 3 (165 eV SID and 90 eV CAD) are very similar to the 565 eV CAD spectrum reported by Barinaga and coworkers [20].

Experiments are in progress to determine the influence of molecular size, collision energy, charge state, and type of surface on the information content of the spectra. Conditions required to produce side-chain cleavage ions of type d and w [21] will also be determined. Peaks corresponding to these ions are present in the SID spectra of singly-charged ions produced by FAB [19], but are not present in the ESI/SID spectra of Figures 1-3.

Conclusions

The spectra reported here show that surface-induced dissociation provides structural information on multiply-charged peptides at sample concentrations compatible with electrospray ionization. The strong similarity between SID and CAD spectra may be the result of partitioning the internal energy to a large number of vibrational modes, such that the effects of different collision energies are not pronounced. Alternatively, multiple sites of protonation within the peptide ion may serve to promote specific fragmentation

pathways. Overall, the results show that surface-induced dissociation is at least as effective as collision-activated dissociation for the structural characterization of multiply-protonated peptides.

Acknowledgements

We would like to thank Kevin Schey and Damon Papac for assistance with the design and operation of the electrospray ionization source and Don Hunt for the use of the triple quadrupole mass spectrometer. This work was supported by the Society for Analytical Chemists of Pittsburgh, a VCU Grant-in-Aid, the Thomas F. and Kate Miller Jeffress Memorial Trust, and the Office of Naval Research.

References

1. Biemann, K. *Biomed. and Environ. Mass Spec.* **1988**, *16*, 99-111.

2. Carr, S.A.; Hemling, M.E.; Bean, M.F.; Roberts, G.D. *Anal. Chem.* **1991**, *63*, 2802-2824.

3. Smith, R.D.; Loo, J.A.; Edmonds, C.G.; Barinaga, C.J.; Udseth, H.R. *Anal. Chem.* **1990**, *62*, 882-899.

4. Fenn, J.B.; Mann, M.; Meng, C.K.; Wong, S.F.; Whitehouse, C.M. *Science* **1989**, *246*, 64-71.

5. Mann, M. *Organic Mass Spectrom.* **1990**, *25*, 575-587.

6. Hamdan, M.; Curcuruto, O. *Int. J. Mass Spectrom. Ion Processes* **1991**, *108*, 93-113.

7. Loo, J.A.; Edmonds, C.G.; Smith, R.D. *Science* **1991**, *248*, 201-204.

8. Loo, J.A.; Edmonds, C.G.; Udseth, H.R.; Smith, R.D. *Anal. Chem. Acta* **1990**, *241*, 167-173.

9. Katta, V.; Chowdhury, S.K.; Chait, B.T. *Anal. Chem.* **1991**, *63*, 174-178.

10. Cooks, R.G.; Amy, J.W.; Bier, M.E.; Schwartz, J.C.; Schey, K.L. *Adv. Mass Spectrom.* **1989**, *11*, 33-52.

11. Bier, M.E.; Schwartz, J.C.; Schey, K.L.; Cooks, R.G. *Int. J. Mass Spectrom. Ion Processes* **1990**, *103*, 1-19.

12. Aberth, W. *Anal. Chem.* **1990**, *62*, 609-611.

13. Williams, E.R.; Henry, K.D.; McLafferty, F.W.; Shabanowitz, J.; Hunt, D.F. *J. Am. Soc. Mass Spectrom.* **1990**, *1*, 413-416.

14. Wysocki, V.H.; Ding, J.-M.; Jones, J.L.; Callahan, J.H.; King, F.L. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 27-32.

15. (a) Hunt, D.F.; Shabanowitz, J.; Martino, P.A.; McCormack, A.L.; Michel, H.; Alexander, J.E.; Sherman, N. *Current Research in Protein Chemistry: Techniques, Structure and Function* Academic Press: New York; 1991; pp. 441-454. (b) Payne, D.M.; Rossomando, A.J.; Martino, P.A., Erikson, A.K.; Her, J.H.; Shabanowitz, J.; Hunt, D.F.; Weber, M.J.; Sturgill, T.W. *EMBO J.* **1991**, *10*, 885-892.

16. (a) Wysocki, V H.; Jones, J.L.; Ding, J.-M *J. Am. Chem. Soc.* **1991**,*113*, 8969-8970. (b) Somogyi, A.; Ding, J.-M.; Kane, T.E.; Wysocki, V.H. manuscript in preparation.

17. Chowdhury, S.K.; Katta, V.; Chait, B.T. *Rapid Comm. Mass Spectrom.* **1990**, *4*, 81-87.

18. Papac, D.I.; Schey, K.L.; Knapp, D.R. *Anal. Chem.* **1991**, *63*, 1658-1660.

19. McCormack, A.L.; Wysocki, V.H. manuscript in preparation.

20. Barinaga, C.J.; Edmonds, C.G.; Udseth, H.R.; Smith, R.D. *Rapid Commun. Mass Spectrom.* **1989**, *3*, 160-164.

21. Johnson, R.S.; Martin, S.A.; Beimann, K. *Int. J. Mass Spectrom. Ion Processes* **1988**, *86*, 137-154.

Figure Legends

Figure 1. Surface-induced dissociation spectrum for the $(M+2H)^{++}$ ion (m/z 649) of angiotensin I at a collision energy of 100 eV (ΔV=50).

Figure 2. (a) Surface-induced dissociation spectrum for the $(M+2H)^{++}$ ion (m/z 881) of renin substrate at a collision energy of 100 eV (ΔV=50). (b) Collision-activated dissociation spectrum for the $(M+2H)^{++}$ ion (m/z 881) of renin substrate at a collision energy of 60 eV (ΔV=30).

Figure 3. (a) Surface-induced dissociation spectrum for the $(M+3H)^{+++}$ ion (m/z 950) of mellitin at a collision energy of 165 eV (Δ=55). (b) Collision-activated dissociation spectrum for the $(M+3H)^{+++}$ ion (m/z 950) of mellitin at a collision energy of 90 eV (ΔV=30).

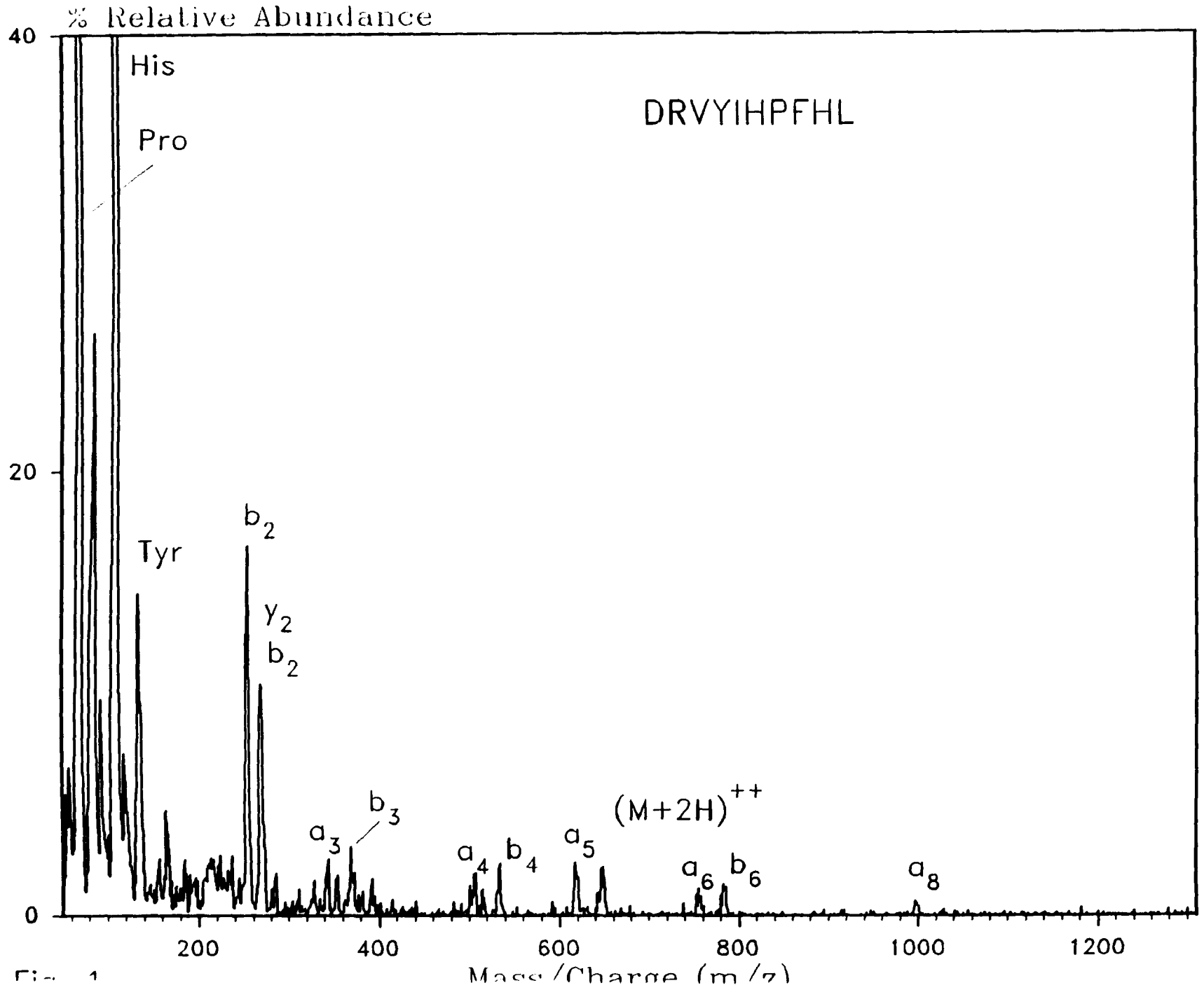

% Relative Abundance
40
20
0
DRVYIHPFHL
His
Pro
Tyr
b_2
y_2
b_2
a_3
b_3
a_4
b_4
a_5
$(M+2H)^{++}$
a_6
b_6
a_8
200
400
600
800
1000
1200
Mass/Charge (m/z)

Fig. 1

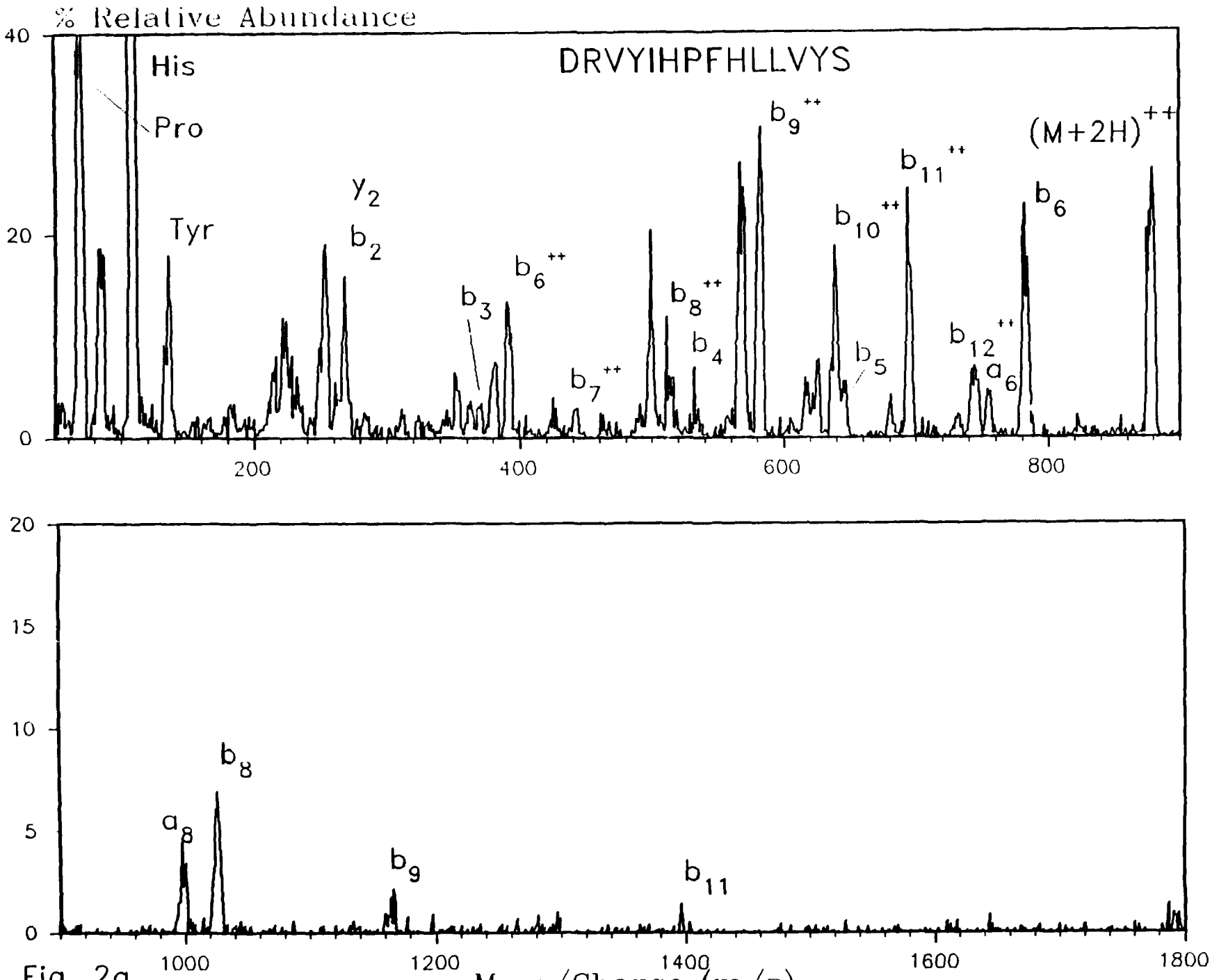

% Relative Abundance
40
20
0
DRVYIHPFHLLVYS
His
Pro
Tyr
y_2
b_2
b_3
b_6^{++}
b_7^{++}
b_8^{++}
b_4
b_9^{++}
b_{10}^{++}
b_5
b_{11}^{++}
b_{12}^{++}
a_6
b_6
$(M+2H)^{++}$
200
400
600
800
20
15
10
5
0
b_8
a_8
b_9
b_{11}
1000
1200
1400
1600
1800
Mass/Charge (m/z)

Fig. 2a

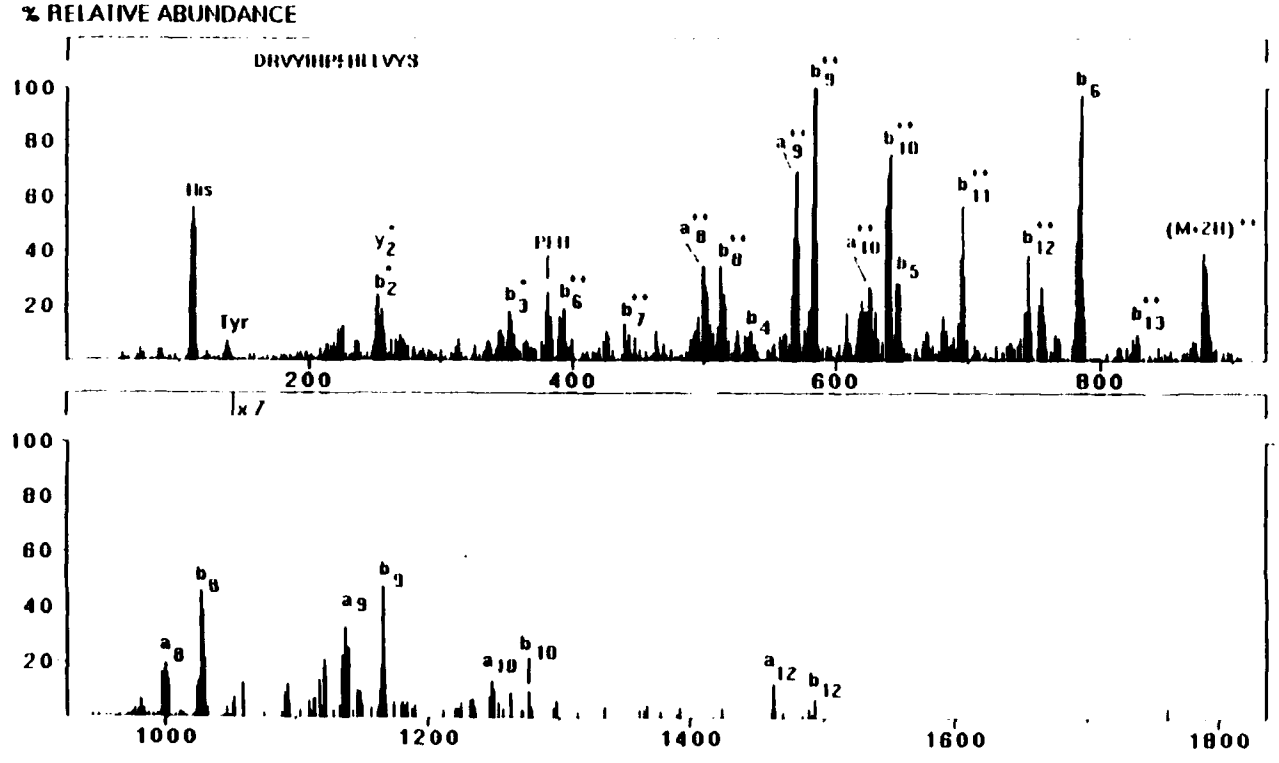

% RELATIVE ABUNDANCE
100
80
60
40
20
His
Tyr
200
400
600
800
x 7
1000
1200
1400
1600
1800

Fig. 2b

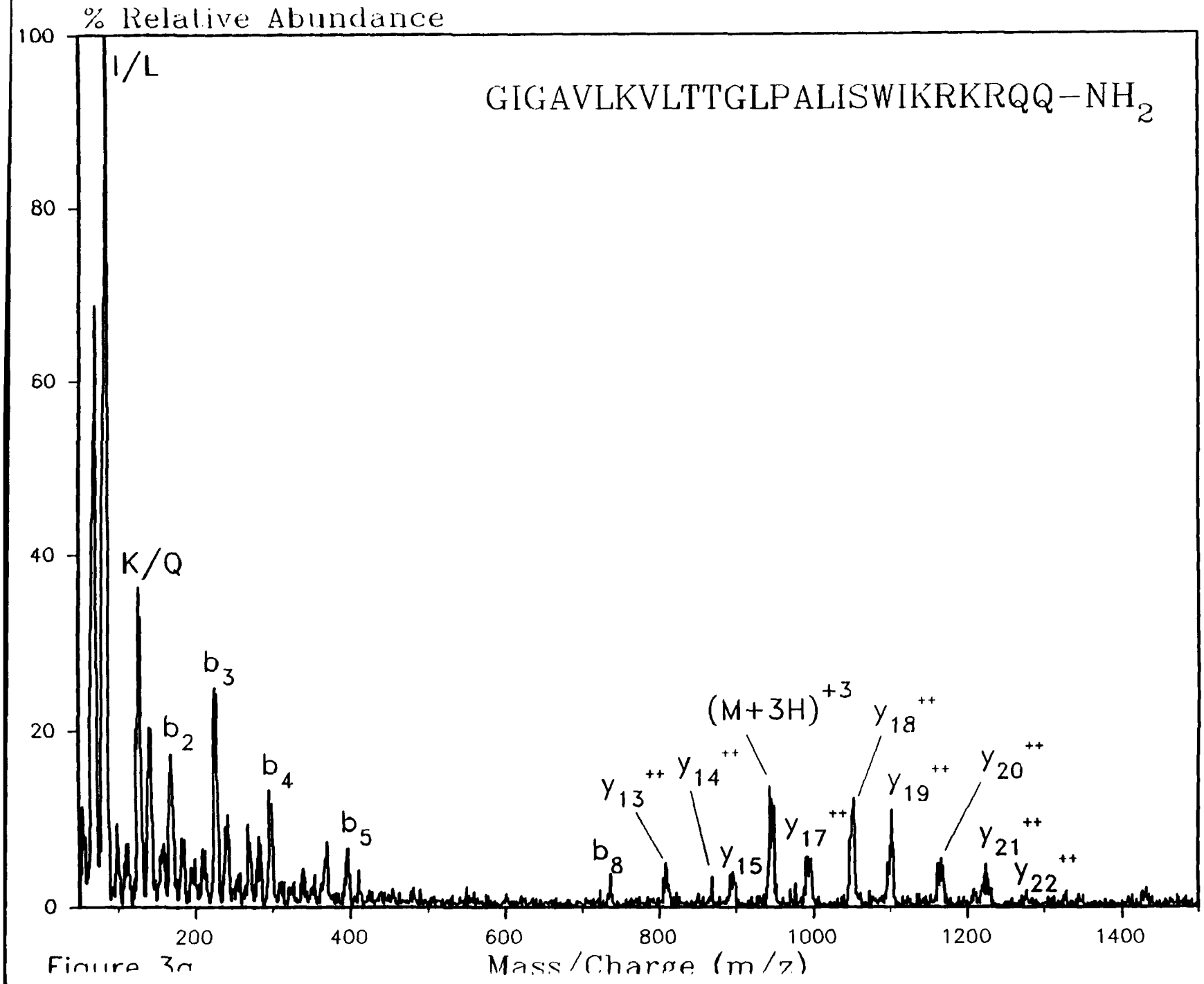

% Relative Abundance
100
80
60
40
20
0
I/L
K/Q
GIGAVLKVLTTGLPALISWIKRKRQQ–NH2
b3
b2
b4
b5
b8
y13++
y14++
y15
(M+3H)+3
y17++
y18++
y19++
y20++
y21++
y22++
200
400
600
800
1000
1200
1400
Mass/Charge (m/z)

Figure 3a

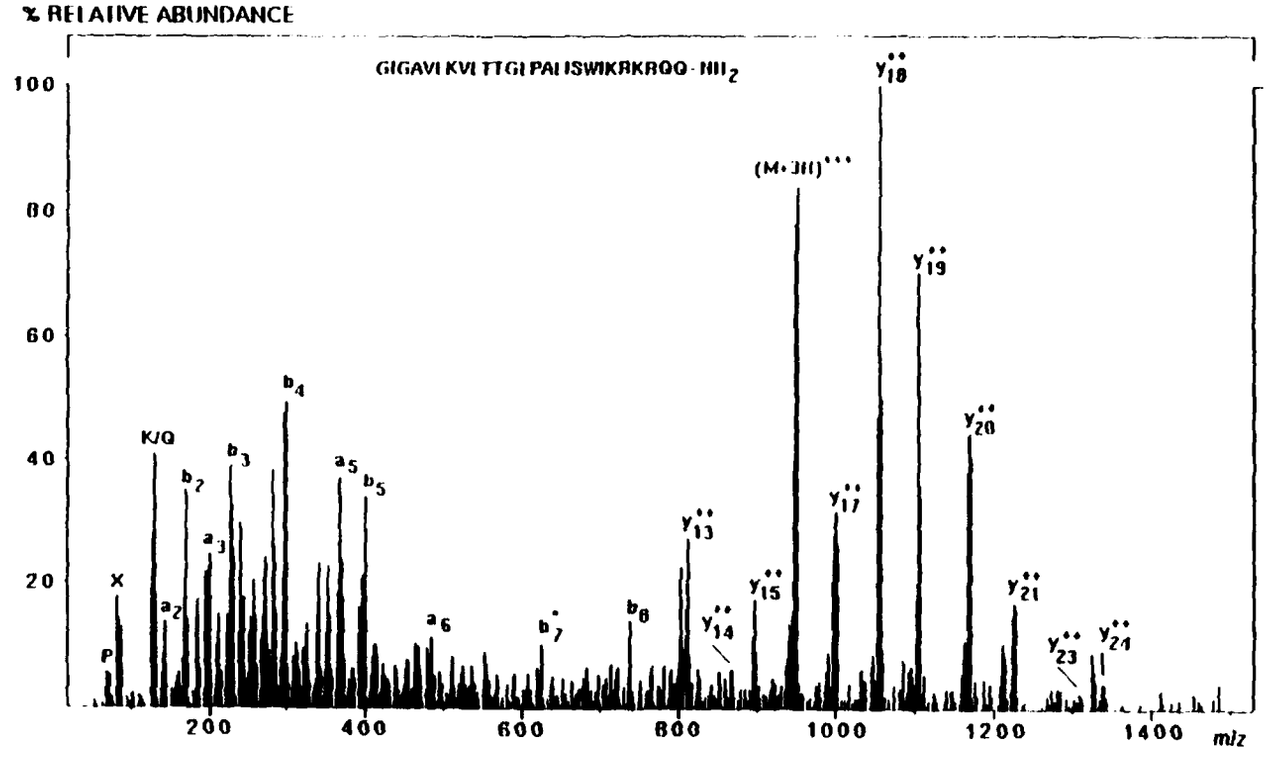

% RELATIVE ABUNDANCE
GIGAVLKVLTTGLPALISWIKRKRQQ-NH2
100
80
60
40
20
200
400
600
800
1000
1200
1400
m/z

Fig. 3b